THE CONSTRUCTION OF FRESH AUTOGENOUS ARTERIAL GRAFTS

I. Use of the Splenic Artery to Bridge a Gap in the Aorta

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ON JAN. 28, 1947, an experimental program was launched in the Surgical Research Laboratory of the Children's Medical Center in Boston which led directly to the first successful use of preserved homologous arterial grafts[1] in human beings by Dr. Robert E. Gross. In the comparatively short space of time that has elapsed since this reinvestigation of the initial work of Carrel and others[2] was instituted, a prodigious amount of effort has been expended in various research centers to solve the many problems that have been raised.

Fresh autogenous venous grafts have satisfied the requirements for bridging gaps in small and medium-sized arteries[3] and for constructing shunts in the surgical relief of portal hypertension[4-6] and superior vena caval obstruction[7] as well as from the aorta to the coronary sinus.[8] Although segments of fresh autogenous inferior vena cava[9-12] and of preserved venous homografts[13] have been employed successfully to bridge gaps in the aorta of the experimental animal, use of this vessel in human beings has not met with enthusiasm. The inaccessibility of the inferior vena cava from the thorax and the high incidence of untoward sequelae following caval ligation[14] have been limiting factors in autotransplants. Rupture of a vein graft in the surgical treatment of an abdominal aneurysm has already been reported,[15] and Johnson and co-workers demonstrated an aneurysmal bulge in an experimental vein graft to the aorta.[10]

Preserved homografts of aorta and large arteries have supplied the most satisfactory solution to date for the problem of bridging a gap in the aorta in human beings. Blood vessel banks have been established in Boston,[16] New York,[17] Denver,[18] and other centers. The major difficulty in maintaining these banks, with the exception of front line military hospitals,[19] has been an inadequate supply of acceptable donor material.

There have been two main lines of investigation to remedy this situation. One of these has been concerned with improved techniques of preservation, so that the safe period of storage of grafts before they must be discarded might be prolonged. These have included refrigeration (Hufnagel,[20, 21] Deterling and associates[22]) and fixation by formalin (Peirce and co-workers[23]), glycerin,[24] and alcohol[25] among others. The second, the possibility of augmenting the supply by sterilization of contaminated grafts by high-voltage cathode ray irradiation, is being explored in the laboratory of Dr. Gross (with human application already accomplished[26]) and by Hui and co-workers.[27]

Functional success, with patent grafts, has thus been achieved with fresh and preserved, venous and arterial, autogenous and homogenous (as well as heterogenous[28]) transplants. The disturbing fact, however, has been that, with

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the exception of the fresh autogenous arterial graft, the cellular constituents of the transplants disintegrate, the muscle cells of the media disappear, and fibrous tissue replacement occurs, often with collagenous degeneration and calcification. Gross[29] has analyzed nineteen cases of coarctation of the aorta in which the narrowed segment had been replaced by a preserved homologous graft. In one patient calcification in the graft has been noted roentgenographically, and in another there is presumptive evidence of thrombosis. While some of the techniques of preservation are conducive to more severe changes than others, in none does the graft present a normal or healthy histologic appearance. Miller and associates[30] have confirmed the previous observations that only in the fresh autogenous graft may one anticipate preservation of the histologic integrity of the media.

The general acceptance at this time of the preserved arterial homograft represents a compromise, with a transplant that might be intact both functionally and anatomically as the ideal. The hope that survival of the cells of a homograft might be enhanced by the adrenocorticotropic preparations has been short-lived. To date, no adequate source of autogenous arterial tissue for fresh aortic grafts has been devised. Recognition of the importance of developing a source of fresh autogenous arterial tissue from which grafts of large diameter might be constructed led to a consideration of the possible use of the splenic artery for this purpose. A long artery of moderate size, associated with an organ that may be sacrificed without jeopardizing the welfare of the subject, supplies the basis for these experiments.

EXPERIMENTAL METHOD

Large mongrel dogs, averaging 50 pounds in weight, were anesthetized with intravenous veterinarian Nembutal. The peritoneal cavity was entered through a liberal left paramedian muscle-splitting incision. The spleen was mobilized and all of its attachments divided, to the pedicle. The tail and body of the pancreas were dissected from the splenic artery, and the artery ligated at as high a level as possible. This usually resulted in a length of available splenic artery measuring from 6 to 10 cm. between the proximal ligature and the hilus of the spleen where the artery breaks up into branches. Autotransfusion with splenic blood was effected by squeezing the spleen, following which the vein was ligated and the spleen resected, with a long pedicle.

At a separate table, the splenic artery was isolated from the vein, and the remainder of the tissue discarded. The artery was split longitudinally, an attempt being made to include any branches in the line of the incision. The opened artery was divided transversely into 3 (or 4) equal segments. The panels thus created were then fashioned into the tubular graft by the steps illustrated in Fig. 1. Each longitudinal suture line consisted of a continuous over-and-over stitch of No. 00000 Deknatel silk, to which petrolatum had been applied, on a small straight atraumatic needle. The saline drip to prevent drying of the tissue was of great importance.

The lower portion of the abdominal aorta was mobilized, usually with division of 1 or 2 pairs of lumbar branches. The aorta was occluded by, and divided between, 2 multitoothed clamps of the Potts type, with resection of a segment up to 1 cm. in length. This gap was bridged by the tubular graft just constructed. The graft was anchored into place at each end. Each circumferential suture line consisted of a continuous everting mattress suture of No. 00000

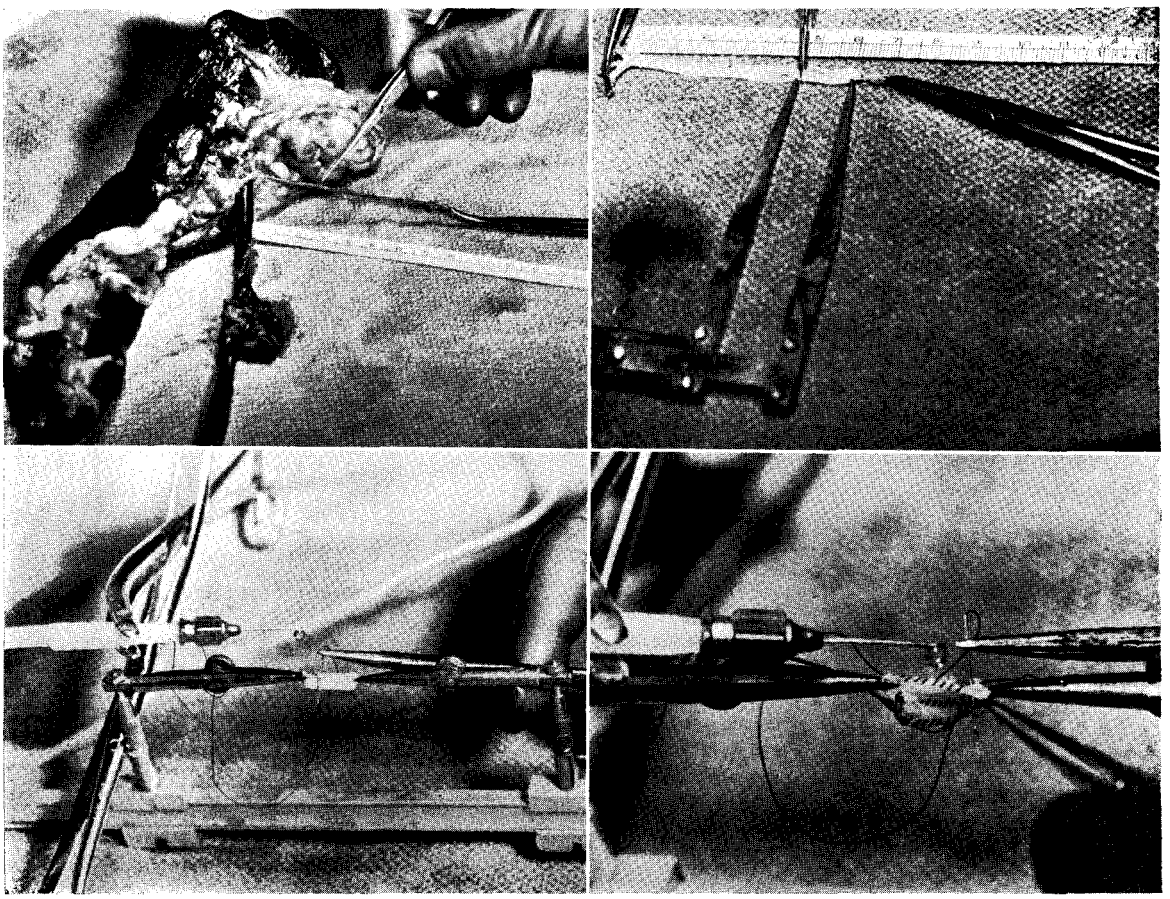


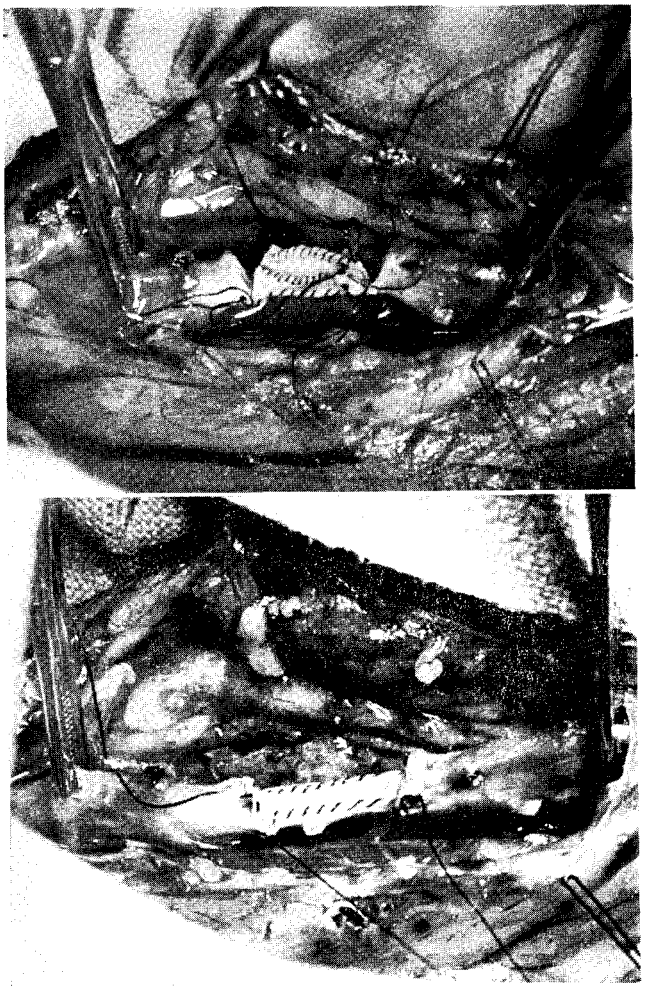
Fig. 1.—Fabrication of tubular graft: *A*, 7 cm. length of splenic artery isolated from hilus and vein; *B*, artery split in long axis, being divided transversely into equal panels; *C*, suture line commenced with first 2 panels; *D*, tubular graft almost completed.

Deknatel silk on a small round atraumatic needle. Care was taken to straddle the end of each longitudinal suture as it was reached. Following completion of the anastomosis, the transplant was irrigated with saline solution introduced by a needle into the aorta, and tested for leaks. Large leaks were repaired by interrupted sutures. The graft was wrapped in compressed Gelfoam,* size 100,

*Supplied through the generosity of The Upjohn Company, Kalamazoo, Mich.

and the distal occluding clamp released, followed by gradual release of the proximal clamp while the graft was gently compressed for several minutes. The Gelfoam usually became bloodstained quickly. When active bleeding had been eliminated, metal dura clips were placed as markers for roentgenographic identification, the posterior parietal peritoneum was united over the graft, and the

A.



B.

Fig. 2.—Fabricated tubular graft sutured into gap in abdominal aorta: *A*, graft anchored to each end of aorta; *B*, second circumferential anastomosis almost complete.

peritoneal cavity was closed in layers. No anticoagulants were used, either locally or systemically. Each dog received an infusion of 1,000 c.c. of 5 per cent glucose in water intravenously during the operation, and 300,000 units of penicillin per day for the first 3 postoperative days (Fig. 2).

After the technique had been acquired, splenectomy and construction of the graft consumed about one hour. By mounting the artery panels in the jig contrived by one of us (A. K.), it was possible for one man to carry out the entire fabrication of the graft alone. While he was thus occupied, the rest of the team mobilized the lower abdominal aorta, from below the renal arteries almost to the aortic trifurcation.

EXPERIMENTAL RESULTS

A total of 30 dogs was utilized for these observations. Technical pitfalls accounted for many early failures. An analysis of the experiments in 3 consecutive groups of 10 each emphasizes the importance of evolving a definitive technique. In the first group of 10 there were 4 good grafts, with only 2 long-term survivors; of the second 10, 6 grafts were satisfactory and 4 dogs did well; in the final 10 there were 8 good grafts with 6 survivors. The discrepancy between the 18 good grafts and 12 long-term survivors is accounted for by 6 postoperative deaths due equally to distemper, aspiration pneumonia, and anesthesia.

Many of the early failures were attributable to necrosis in the graft due to excessive pressure by the jig holders; the placing of the sutures in the fabrication of the graft in such a way that too much or too little tissue was included, or strands of adventitia inadvertently carried along; drying of the panels; improper construction of the anastomotic suture lines; and the lack of Gelfoam. The completed technique included careful adjustment of the jig holders, meticulous placing of all sutures, a continuous saline drip to keep the panels moist during fabrication of the graft, and a backing for the graft of compressed Gelfoam. By observing all of these details, there was only 1 unsatisfactory experiment in the last 8 consecutive dogs, and that was due to clumsiness on the part of the operator in performing the anastomosis.

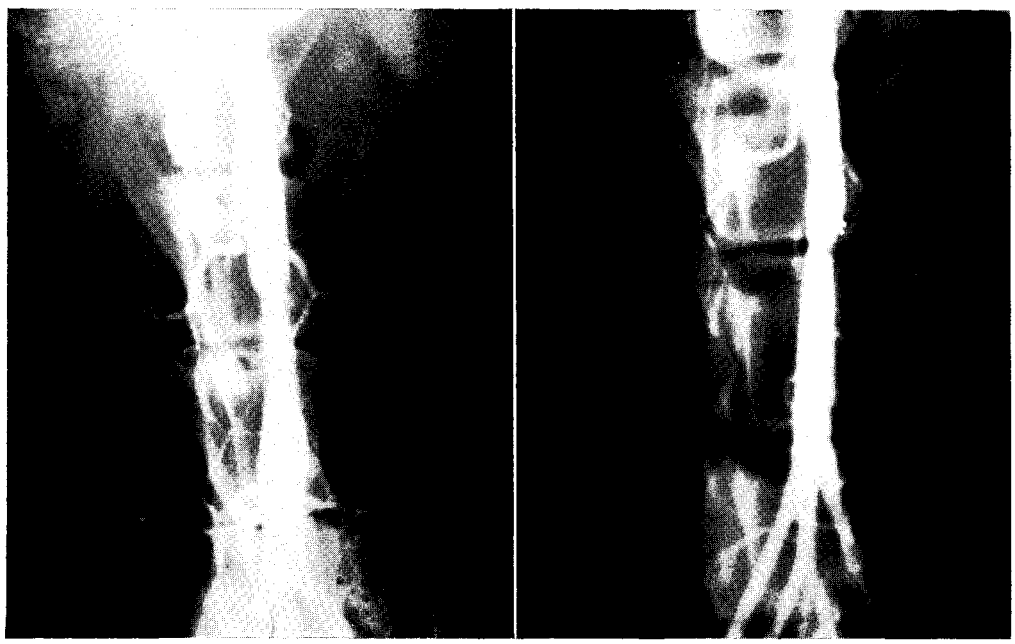
The average diameter of the pulsating abdominal aorta measured 11 mm., and that of the splenic artery 3 mm. The 3 panel grafts employed in the early experiments were uniformly smaller in caliber than the aorta, but the 4 panel grafts employed later made a nice fit. There was usually a sufficient length of splenic artery available to permit construction of a tubular graft up to 2 cm. long. In only 1 instance was the splenic artery so thin and narrow that a satisfactory graft was not created. Pancreatitis following mobilization of the splenic artery occurred only once, with acute gastric dilatation leading to the death of the animal.

Hemorrhage from rupture of the graft occurred 6 times in the first 14 experiments, and this was eliminated as the technique became standardized. There were 4 instances of thrombosis of the graft, of which 1 was associated with infection and the other 3 with the traumatic causes already discussed.

Use of the splenic artery in 30 dogs thus resulted in 18 satisfactory aortic grafts. Of the 12 failures, 11 were due to avoidable factors that were eliminated as the experiments progressed, and in only 1 dog was the artery itself unsuitable for the fabrication of a tubular graft.

Functional evaluation of the grafts has been based upon the postoperative course and upon aortography. Retrograde aortagrams were made soon after operation and before sacrifice on every animal. In no case had the caliber of the graft lessened in the interval between examinations, the longest-term survivor to be sacrificed to date having survived 6 months. Aortography was done by injecting 30 c.c. of 35 per cent Diodrast rapidly in retrograde fashion into a cannulated femoral artery and taking rapid films with the Fairchild 9½ by 9½ inch magazine roll camera (Fig. 3).

Anatomical evaluation of the transplants was based on gross and histologic observations. In all the specimens examined within 4 months after operation, there was a distinct difference between the color of the intima of the graft and



A. *B.*

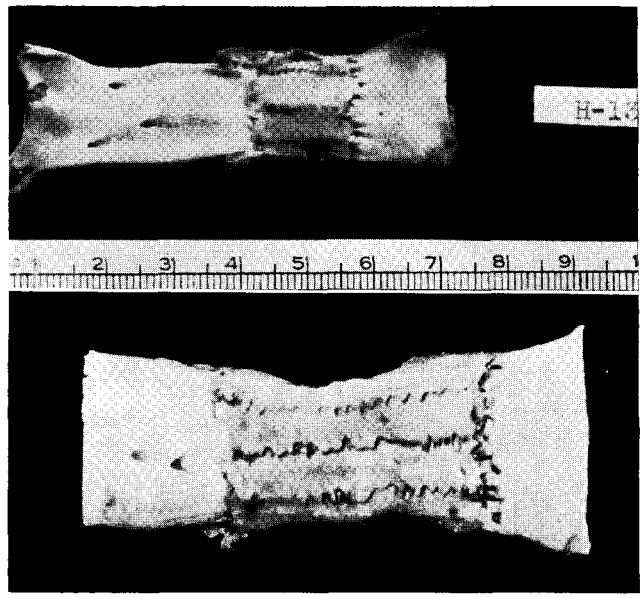
Fig. 3.—Retrograde aortagrams 2 weeks after insertion of *A*, 3 panel, and *B*, 4 panel arterial autografts.

that of the host aorta. A tan in the early specimens was gradually replaced by a pearly gray in the older ones, so that by 6 months after operation the color of the intima was uniform. In dogs dying within the first 10 days after operation the intimal surface was shaggy, and the sutures bare. Small flat mural thrombi were not uncommon, more often attached to the graft panels than to the sutures. The importance of the speed and force of blood flow in the aorta in minimizing thrombus formation cannot be overemphasized. By the tenth postoperative day the circumferential sutures and the ends of the longitudinal sutures were partly covered by endothelium in 2 specimens, with bare areas in the central portions of the longitudinal sutures and a few tiny shallow ulcerations in the panels. Two additional specimens examined on the fifteenth and

sixteenth days, respectively, showed almost complete smooth covering of the sutures, with small shaggy patches in the central part of the graft. Studies have been conducted on 5 grafts examined at 2, 3, 4 (2 dogs) and 6 months after insertion. No thrombi or ulcerations were present, and the intimal surface of the transplants, including the suture lines, was completely covered by endothelium. Straightening of parts of the longitudinal suture lines in the six-month specimen indicates that some of the loops have pulled out of the panels in these areas; the silk is still covered by endothelium (Fig. 4). Additional dogs are being maintained for long-term survival observations.

The thickness of the graft, which at the time of insertion was considerably less than that of the aorta, gradually increased with the organization of the Gelfoam, passing through a stage of excessive thickness to a final state in which the walls of host and graft had approximately equal measurement.

A.



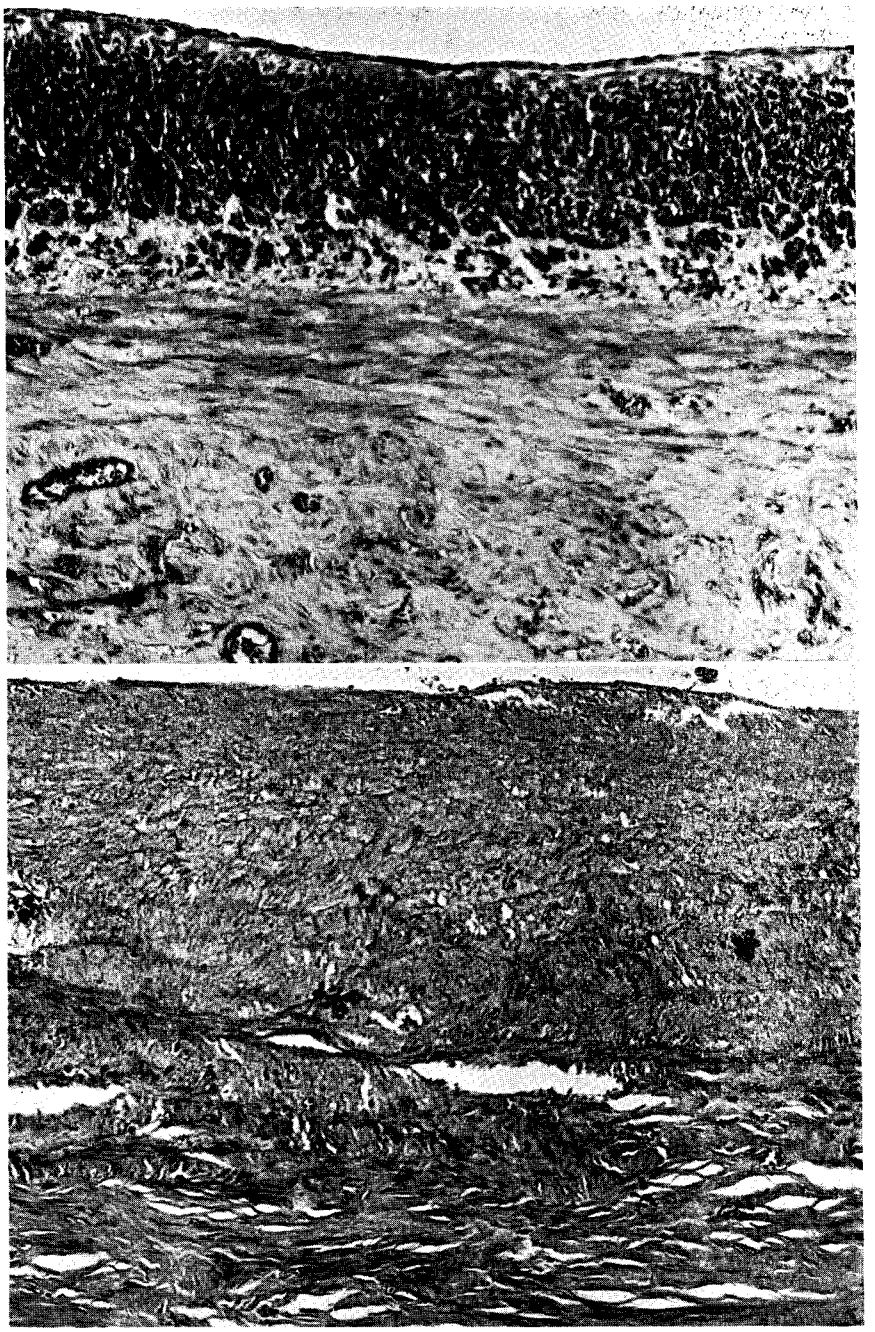
B.

Fig. 4.—Gross appearance of fabricated tubular grafts *A*, 65, and *B*, 195 days after implantation. Suture lines are smoothly covered by endothelium, and partly pulled straight in *B*.

The histologic appearance of these grafts constitutes the crux of the entire study. The very thin intimal layer of the original splenic artery was replaced by a fine endothelial covering resting on a fairly dense layer of connective tissue. The subendothelial fibrosis varied markedly from 1 specimen to another, and in different parts of the same specimen; it may represent, in part, organization of blood clot. The anastomotic junction was occupied by a wedge of fibrous tissue with a thin overlying endothelial layer.

Preservation of the integrity of the media was the most striking characteristic of the grafts. In animals sacrificed at 2 months, 4 months, and at 6

A.



B.

Fig. 5.—*A*, Survival of media of fresh arterial autograft, postoperative 137 days. *B*, Collagenous degeneration of media of fresh aortic homograft, postoperative 83 days. (Masson stain, original ×180.)

months the thickness of the media was the same as in the original splenic artery. A slight to moderate increase in connective tissue was revealed by the Masson stain, but survival of most of the muscle cells of the media was the rule. This is in direct contrast to the fate of the media in homografts, where collagenous

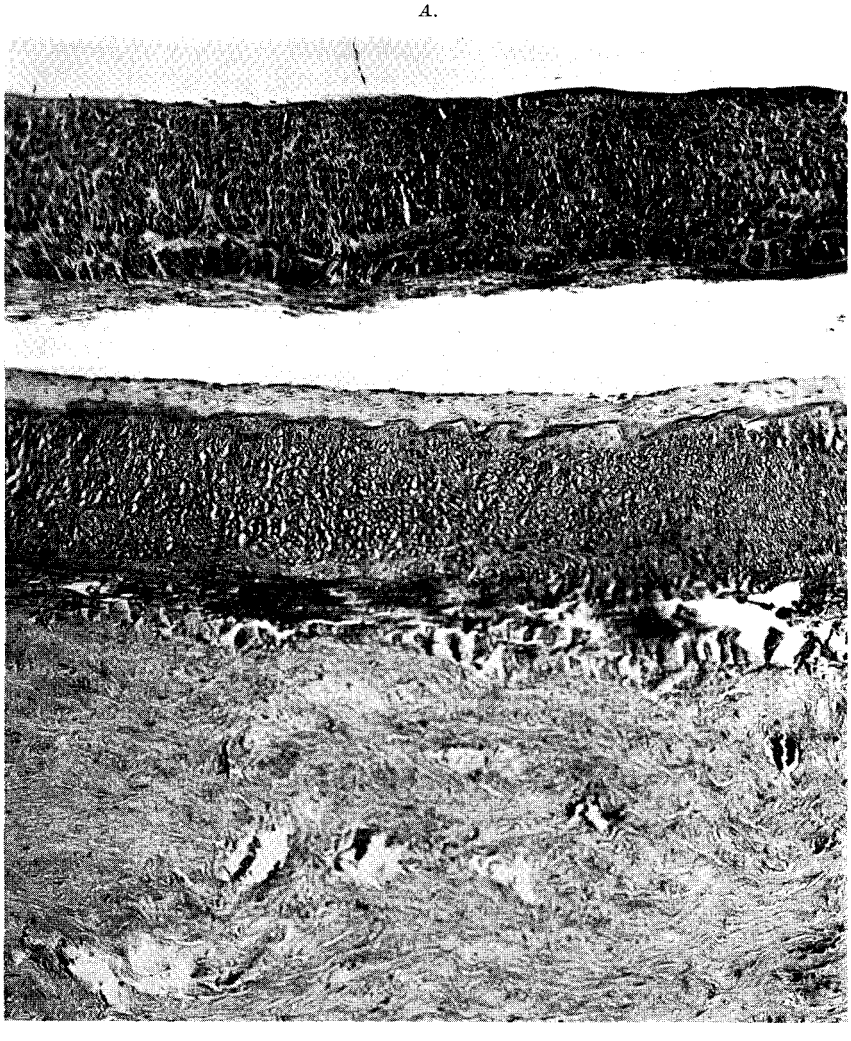


Fig. 6.—*A*, Normal splenic artery, and *B*, splenic artery—aortic graft 195 days after insertion. Note subendothelial fibrosis, survival of media, and extensive thickening of adventitia (organized Gelfoam). (Masson stain, original ×180.)

degeneration and connective tissue replacement occur uniformly. Fibrillation and thinning of the elastic tissue laminae were also less in the autografts than in the homografts (Fig. 5).

In 2 dogs, sacrificed 3 and 4 months postoperatively, it was noted that the integrity of the media was distorted to a far greater degree than in the other

specimens. The thickness of the media was considerably diminished, and there was a substantial reduction in the number of surviving muscle cells. In these specimens also the intimal layer was much thicker than in the others, suggesting a more intense reaction, possibly traumatic. Compromised survival of the media in these specimens may have been on the basis of compression between thick intimal and dense adventitial layers. This suggestion receives support from study of adjacent areas in a 3 month transplant: the media is intact beneath a thin intima, and markedly changed in the region of a thick intima. Nourishment of the innermost layers of the media by oxygenated blood in the lumen of the vessel is also indicated by a comparison of these two zones, in which the adventitia is of uniform thickness and the appearance of the media is correlated with changes in the intima.

A remarkable transformation of the thin adventitia of the graft to a thick dense layer of connective tissue accompanied the organization of the Gelfoam. This was in progress in a 2 month specimen, and complete at 3 months. The newly formed outer wall was liberally supplied with blood vessels in all of the specimens (Fig. 6).

The homologous transplants utilized for comparison with this group of fabricated autografts were obtained from a series of aortic grafts carried out by one of us (E. S. H.) with Dr. Robert E. Gross in the surgical research laboratory of the Children's Hospital, Boston, in 1947. Resection of a segment of the abdominal aorta in dogs was performed under the same conditions as in the present experiments. The gap was bridged by a fresh segment of aorta taken from donor dogs in 10 animals, with 2 deaths. Transplantation of aortic segments preserved for 6 hours in Ringer's solution at ordinary icebox temperatures was performed 9 times, with 2 deaths. The technique of anastomosis was constant in all the operations. The dogs were sacrificed after periods ranging from 2 months to 1 year postoperatively; in each series there were 5 grafts classified as good to excellent. The degeneration of the media and replacement by fibrous tissue in these specimens conform to the biologic behavior pattern described by others for fresh and preserved homotransplants.[2, 10, 11, 31-34]

DISCUSSION

At the third annual meeting of the Society for Vascular Surgery in Atlantic City on June 5, 1949, Dr. Julian Johnson and his co-workers[9] presented the use of autogenous femoral vein grafts to construct shunts in 2 patients with cyanotic heart disease. In the discussion which followed this paper it was stated by Dr. Gross, and generally agreed, that the repair of blood vessel defects should be accomplished by direct end-to-end approximation of the vessel, by a fresh autogenous graft, or by a preserved homograft, in that order of preference. The lack of availability of arterial tissue has prevented the use of fresh autogenous arterial grafts for bridging gaps in the aorta. This was reemphasized by various discussants at the seventy-first annual meeting of the American Surgical Association in April, 1951.[12]

If the histologic integrity of the media of a graft is of importance—and it is difficult to think that it would be unimportant—this demonstration of the

feasibility of constructing autogenous arterial grafts may have potential clinical application. In a series of observations based on 17 autopsies on adult human beings in the Pathology Laboratory of the Montefiore Hospital,* the circumference of the splenic artery measured 1 to 1.5 cm. in 68 per cent of the examinations, the circumference of the thoracic aorta measured 5 to 7 cm. in 71 per cent, and that of the abdominal aorta 4 to 5 cm. meters in 60 per cent. Approximately 10 to 12 cm. of splenic artery were estimated as being surgically accessible. These relative measurements are comparable to those in the dog, and indicate the suitability of this tissue for aortic grafts. The splenic artery is accessible from either the abdomen or the left side of the chest.

The observations made in these experiments would tend to confirm the concept that the intimal surface of the graft is replaced by cellular elements from the host, and that the ingrowth of endothelium commences from the host at each end of the graft. This seems more likely than the formation of endothelium from the organization of blood clot in situ as suggested by Wylie and associates.[35] Support is also lent to the impression of Sako and co-workers[36] that the longitudinal suture lines may act as struts along which the growth of endothelium may be accelerated. These authors transplanted tubular grafts, constructed from pericardium and from fascia, between the divided ends of the thoracic aorta in dogs. Thickening and dilatation of the transplants occurred, with the deposition of atheromatous material.

The density of the newly formed adventitia, resulting from organization of the Gelfoam, compared favorably with that described by Wylie[35] following the use of fascia lata as a support around weakened arteries. The importance of the adventitia in terms of the bursting strength of an artery has been documented by Winternitz and co-workers.[37] Organization of the Gelfoam in these experiments suggests the usefulness of this material in reinforcing vascular suture lines, transplants, and blood vessels that have been subjected to surgery. This behavior of Gelfoam contrasts with the persistence of the substance in the intracranial cavity, where it has been found almost unchanged as long as 2 years after insertion.[38]

The growth of anastomotic suture lines[39] and of fresh autogenous aortic and vena caval grafts has been demonstrated.[10, 11] Growth of the fabricated autograft is under investigation. It is not suggested that the splenic artery affords the only or the best source of tissue for a fabricated autogenous arterial graft. Excessive tortuosity and advanced atherosclerosis may eliminate this vessel at times. Other sources of arterial tissue merit consideration.

SUMMARY

1. A technique has been devised for the fabrication of fresh autogenous arterial transplants from segments of the splenic artery.

2. Tubular grafts may be fashioned to any required diameter.

3. Grafts constructed from segments of the splenic artery have been used to bridge a gap in the abdominal aorta in a series of dogs.

*By Dr. Stanley Altman.

4. An adequate functional result has been obtained, with long-term patency of the grafts and survival of the animals.

5. Preliminary histologic observations, in survival studies up to 6 months postoperatively, indicate preservation of the integrity and survival of the muscle cells in the media of the grafts.

6. These fresh autogenous arterial grafts are functionally equal to, and anatomically superior to, aortic homografts.

7. The disadvantages of splenectomy and the time required to construct the graft are offset in part by eliminating the problems of supply, sterilization, preservation, and the biologic specificity of tissue proteins.

8. Fresh autogenous vein grafts are ideal for bridging gaps in arteries of small to moderate size. The use of fabricated fresh autogenous arterial grafts is recommended for defects involving the aorta.

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